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Lycopene and the LXRα agonist T0901317 synergistically inhibit the proliferation of androgen-independent prostate cancer cells via the PPARγ-LXRα-ABCA1 pathway☆

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Abstract

In our previous study, we demonstrated that lycopene can inhibit the proliferation of androgen-dependent prostate LNCaP cancer cells through the activation of the peroxisome proliferator-activated receptor gamma (PPARγ)–liver X receptor alpha (LXRα)–ATP-binding cassette transporter 1 (ABCA1) pathway. However, it is still unclear whether lycopene possesses similar effects in androgen-independent prostate cancer cells DU145 and PC-3. As lycopene inhibited the proliferation of both cell types to a similar extent, we chose DU145 cells for most of the subsequent studies. We show that lycopene significantly increased protein and mRNA expression of PPARγ, LXRα and ABCA1 and cholesterol efflux (i.e., decreased cellular cholesterol and increased cholesterol in culture medium). Lycopene (10 μM) in the presence of a specific antagonist of PPARγ (GW9662) or of LXRα (GGPP) restored the proliferation of DU145 cells and significantly suppressed lycopene-induced protein and mRNA expression of PPARγ and LXRα and cholesterol efflux. Liver X receptor α knockdown by siRNA against LXRα significantly promoted the proliferation of DU145 cells, whereas si-LXRα knockdown followed by incubation with lycopene (10 μM) restored the proliferation to the control level. Furthermore, lycopene in combination with the LXRα agonist T0901317 exhibited synergistic effects on cell proliferation and protein expression of PPARγ, LXRα and ABCA1. These results demonstrate that lycopene can inhibit DU145 cell proliferation via PPARγ-LXRα-ABCA1 pathway and that lycopene and T0901317 exhibit synergistic effects.

Keywords: ABCA1; LXRα; Lycopene; PPARγ; Prostate cancer

1. Introduction

Prostate cancer is one of the most common diagnosed malignancy and is the second cause of cancer death in American men, with 217,730 new cases and 32,050 deaths during 2010 [1]. Although the etiology of prostate cancer is still unclear, several risk factors are well known for prostate cancer such as race, age, socioeconomic position, dietary factor and family history of the disease [1,2]. LNCaP, PC-3 and DU145 are the most commonly used prostate cancer cell lines [3]; among them, LNCaP cells are androgen dependent, whereas DU145 and PC-3 cells are androgen independent and are isolated from human prostatic adenocarcinoma metastatic to bone [4] and brain [5].

Lycopene, a major dietary and serum carotenoid, contains 11 conjugated and 2 nonconjugated double bonds [6,7]. Epidemiological studies have suggested that higher intakes of lycopene are related to a reduced risk of several types of cancer, such as prostate cancer [8,9]. Much evidence indicates that lycopene exhibits multiple biological functions, such as antioxidant activity [10], antimetastasis [11–13], antiangiogenesis [11], anti-inflammatory ability [9] and anticancer [14–16]. Lycopene also inhibits the growth of prostate tumor cell lines DU145 and PC-3 *in vivo* [17–19]. Several possible mechanisms are involved in the anticancer action of lycopene in prostate cancer cells, including induction of apoptosis [14], cell cycle arrest [15], alternation of mevalonate pathway and Ras signaling [20].

Nuclear hormone receptors, such as peroxisome proliferator-activator receptors (PPARs), liver X receptor (LXR) and pregnane X receptor, are responsible for inducing transcriptional activity by binding with lipophilic hormones such as steroid and thyroid hormones [21]. The PPAR family consists of three subtypes: PPARα, PPARβ/δ and PPARγ [22]; among them, PPARγ is the most widely studied. Activation of PPARγ with specific ligands, such as troglitazone and 15-deoxy-Delta (12,14)-prostaglandin J(2), inhibits the growth of several types of cancer cells, such as prostate cancer, breast cancer and lung cancer [23–25]. Liver X receptors comprise two subtypes, LXRα and LXRβ, which are activated by oxysterols, such as 22(R)- and 24(S)-hydroxycholesterol, and are involved in central transcriptional regulator for lipid homeostasis [26]. Liver X receptor α is expressed at high levels in the liver, intestine, adipose tissue and macrophages, whereas LXRβ is expressed ubiquitously [27].

Several lines of evidence have indicated that the activation of nuclear receptors, such as PPARγ and LXRα, is related to reducing growth of prostate cancer [23,28]. Campbell et al. have indicated that lycopene consumption increases PPARγ mRNA expression in prostate tissue of F344 rats [29]. We recently showed that lycopene can exert its antiproliferative effect through activation of PPARγ-LXRα-ABCA1

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pathway in androgen-dependent prostate cancer cells (LNCaP) [30]. However, it is unclear whether lycopene exerts similar effects in androgen-independent prostate cancer cells. This question was examined in the present study. In addition, we investigated the possible synergistic effects of lycopene and the LXRα agonist T0901317.

2. Materials and methods

2.1. Chemicals and lycopene preparation

All chemicals used are of the highest grade. Tetrahydrofuran (THF) containing 0.0025% butylated hydroxytoluene (BHT) was obtained from Merck (Darmstadt, Germany). Dulbecco's modified Eagle's medium (DMEM), RPMI1640, nonessential amino acid, penicillin/streptomycin, sodium pyruvate, fetal bovine serum (FBS) and trypsin were obtained from Gibco/BRL (Grand Island, NY, USA). Lycopene was purchased from Wako (Japan). The purity of commercial lycopene was approximately 98%, as claimed by the supplier (Wako, Japan) and confirmed in our laboratory [12,13]. Lycopene was soluble in THF/BHT to form a 10-mM stock solution, which was diluted with THF at indicated ratios (1:1, 1:3, 1:7) and then diluted with FBS at indicated ratio (1:9) [31]. Tetrahydrofuran/BHT-FBS-lycopene were added to the culture medium at a calculated final concentration of 2.5–20 μM. Tetrahydrofuran at 0.2% (vol/vol) and FBS at 1.8% (vol/vol) served as the solvent for lycopene, which did not significantly affect the assays described below.

2.2. Cell culture and cell proliferation assay

DU145 cells (Bioresource Collection and Research Center, BCRC: 60348) and PC-3 cells (BCRC 60122) were purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan). The cells were grown in DMEM or RPMI1640 medium containing 10% (vol/vol) FBS, 0.37% (wt/vol) $NaHCO_3$, penicillin (100 U/ml) and streptomycin (100 U/ml) in a humidified incubator under 5% CO_2 and 95% air at 37°C. Cell proliferation was determined using [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] (MTT) assay. Briefly, the cells were cultured in 24-well plates at 5×10^4 cells/well in DMEM or RPMI1640 for 24 h. Then, the cells were treated with lycopene at various concentrations (0–20 μM) for various periods of time (12, 24 and 48 h). After the exposure period, the medium was removed, and the cells were washed with PBS followed by incubated with MTT solution for 1 h. The medium was discarded, and the formazan was solubilized in dimethyl sulfoxide and measured spectrophotometrically at 570 nm. The percentage of viable cells was estimated by comparing with untreated control cells.

2.3. Western blotting

Cells were incubated with 0–20 μM lycopene at 37°C for 12, 24 and 48 h. After the medium was removed, cells were lysed with radio-immunoprecipitation assay (RIPA) buffer containing proteinase inhibitors. The cell lysate were scraped, followed by centrifugation for 30 min at 4°C. A portion of the supernatant (containing 50 μg protein) was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto a polyvinylidene fluoride (PVDF) membrane. After blocking with tris buffered saline (TBS) buffer (20 mM Tris–HCl, 150 mM NaCl, pH 7.4) containing 5% nonfat milk for 1 h, the membrane was incubated with PPARγ, LXRα and ABCA1 antibody (Santa Cruz Biotechnology, Inc.) overnight and then incubated with horseradish-peroxidase-conjugated antimouse IgG for 1 h, followed by visualization using an ECL chemiluminescent detection kit (Amersham Co., Bucks, UK). The relative protein levels were quantitated by Matrox Inspector 2.1 software.

2.4. Reverse transcriptase polymerase chain reaction (RNA isolation and sequencing)

Total cellular RNA was isolated from cell culture (RNAzol kit) and reverse transcribed into cDNA (MMLVReverse Transcriptase, Gibco/BRL) using oligo (dT) as primers and then amplified with primer bases on PPARγ, LXRα, ABCA1 and β-actin (internal control) sequences. The primers for amplifying PPARγ cDNA were 5′-TCTCCAGCATTTCTACTCCAC-3′, located in the 5′-untranslated region, and 5′-GCCAA-CAGCTTCTCCTTCTCG-3′, located in the 3′-untranslated region. The primers for amplifying LXRα cDNA were 5′-TCAGCCGGGAGGACCAGATTG-3′ and 5′-CCGGAGGCT-CACCAGTTTCATTAG-3′. The primers for amplifying ABCA1 cDNA were 5′-ACAAC-CAAACCTCACACTACTG-3′ and 5′-ATAGATCCCATTACAGACAGCG-3′. The primers for amplifying β-actin were 5′-GAGCGGGAAATCGTGCGTGAC-3′ and 5′-GCCTAGAAG-CATTTGCGGTGGAC-3′. Polymerase chain reaction (PCR) amplification was performed with a thermal cycler as follows: denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 90 s (35 cycles), followed by a final incubation at 72°C for 7 min. The sizes of the amplification products of PPARγ, LXRα, ABCA1 and β-actin were 460, 404, 439 and 518 bp. The PCR products were added to 6× staining buffer (EZVISION THREE DNA DYE & BUFFER, Amresco, USA) and were subjected to 1% agarose gel electrophoresis. Matrox Inspector 2.1 software was used to quantify the relative level of PPARγ, LXRα and ABCA1.

2.5. Cholesterol loading and efflux assay

After incubation, DU145 cells were lysed with RIPA buffer containing proteinase inhibitors. Then, the cell lysate and the culture medium were collected and processed; total cholesterol was quantified by Cholesterol/Cholesterol Ester Quantitation Kit (BioVision). The assay was performed in triplicate by following the instructions provided by the manufacturer.

2.6. Transient transfection of siRNA against LXRα

The plasmids containing siRNA against LXRα (si-LXRα; ON-TARGETplus SMART pool), nontargeting siRNA (si-control) and DharmaFECT transfection reagent were purchased from Thermo Scientific Dharmacon (USA). Si-LXRα and si-control stock solutions (20 μM) were diluted with DEPC water to form 5-μM solutions. DharmaFECT transfection reagent was mixed with 5 μM si-LXRα or si-control incubated for 20 min and then added to the culture medium at 25 nM. The cells were incubated with si-LXRα and si-control for 48 h.

2.7. Statistical analyses and calculation of synergistic effect

Values are presented as means±S.D. and analyzed using one-way analysis of variance, followed by least significant difference (LSD) test for comparisons of group means. $P<.05$ was considered significant. The statistical analysis was performed using SPSS for Windows, version 10 (SPSS, Inc., Chicago, IL, USA). The synergy of data is calculated as [(lycopene +T0901317)−control] ÷ [(lycopene−control)+(T0901317−control)] [32]. According to this formula, a value greater than 1.0 is synergistic, a value of 0.5–1.0 is additive, while a value less than 0.5 is antagonistic [33].

3. Results

3.1. Lycopene inhibits the cell proliferation in DU145 and PC-3 cells

As expected, incubation of DU145 cells with lycopene (2.5–20 μM) for 12, 24 and 48 h markedly decreased the cell proliferation, and the inhibitory effect of lycopene increased with increasing concentration up to 10 μM, whereas the effect of 20 μM lycopene was lower than that of 10 μM (Fig. 1). Similarly, lycopene also significantly inhibited the cell proliferation of PC-3 cells during 48-h incubation (Supplementary Fig. 1).

3.2. Effects of lycopene on the protein and mRNA expression of PPARγ, LXRα and ABCA1 as well as cholesterol efflux in DU145 cells

Based on the cell proliferation data, we chose the highest inhibitory concentration of lycopene (10 μM) to examine the time-dependent effect of lycopene on protein and mRNA expression of PPARγ, LXRα and ABCA1 and cholesterol efflux in DU145 cells for 12, 24 and 48 h. Results reveal that lycopene significantly increased the protein and mRNA expression of PPARγ, LXRα and ABCA1 during 48-h incubation, and the effect was strongest at 24-h but weakened at 48-h incubation (Fig. 2A and C). Similarly, lycopene significantly increased the level of

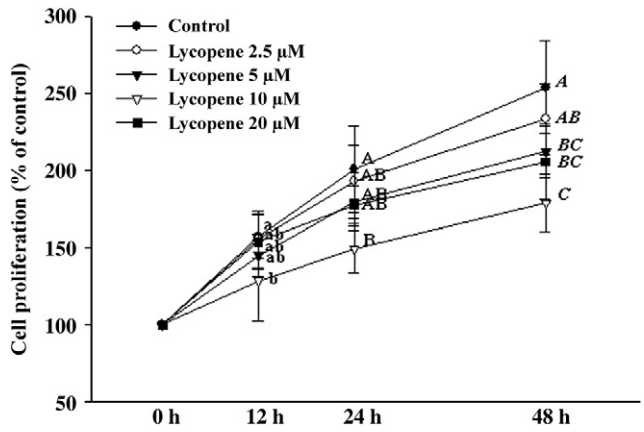

Fig. 1. Effect of lycopene (0–20 μM) on cell proliferation in DU145 prostate cancer cells after incubation for 12, 24 and 48 h. Values are means±S.D., $n=3$; means from the same incubation time not sharing a letter differ significantly ($P<.05$).

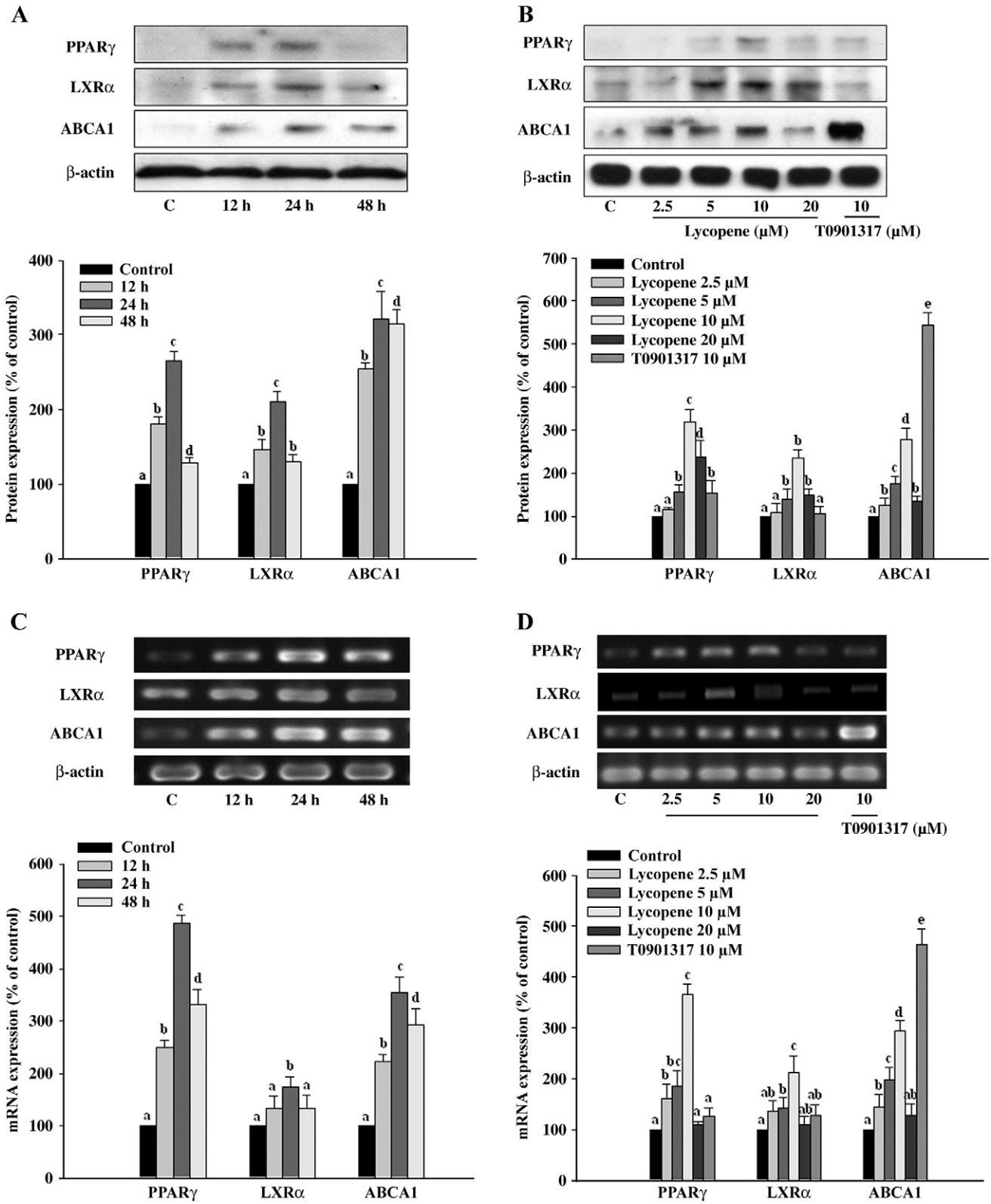

Fig. 2. Time course and concentration effects of lycopene on the expression of PPARγ, LXRα and ABCA1 in DU145 cells. (A) Protein expression in cells incubated with 10 μM lycopene for 12, 24 and 48 h; (B) protein expression in cells incubated with 0–20 μM lycopene for 24 h; (C) mRNA expression in cells incubated with 10 μM lycopene for 12, 24 and 48 h; and (D) mRNA expression in cells incubated with 0–20 mM lycopene for 24 h. T0901317 (10 μM) serves as positive control. Values are means±S.D., $n=3$; means from the same incubation time not sharing a letter differ significantly ($P<.05$).

cholesterol in the culture medium but significantly decreased the level of cellular total cholesterol, and this effect was also strongest at 24-h incubation (Fig. 3A).

Based on the time course results, we chose the most effective time (24-h incubation) to investigate the concentration effect of lycopene (0–20 μM) on protein and mRNA expression of PPARγ, LXRα and ABCA1 and cholesterol efflux in DU145 cells. We found that lycopene significantly increased the protein and mRNA expression of PPARγ, LXRα and ABCA1 and that the effect of lycopene increased with increasing concentration up to 10 μM but somewhat weakened at 20 μM (Fig. 2B and D). In addition, lycopene significantly increased the level of cholesterol in the culture medium, but significantly decreased the level of cellular total cholesterol at 24 h in DU145 cells (Fig. 3B). Using T0901317, an LXR agonist, as positive control, we found that the agonist did not significantly affect the LXRα expression but markedly increased the expression of ABCA1 in DU145 cells (Fig. 2B and D). Similar results were obtained when PC-3 cells were used (Supplementary Fig. 2).

3.3. Effect of lycopene in combination with a specific antagonist of PPARγ (GW9662) or of LXRα (GGPP) on proliferation, PPARγ and LXRα protein expression as well as cholesterol efflux in DU145 cells

To confirm the involvement of the PPARγ-LXRα-ABCA1 pathway, we incubated DU145 cells with lycopene (10μM) in the presence or absence of a selective antagonist of PPARγ (GW9662, 10 μM) or of LXRα (GGPP, 10 μM) for 24 h. As shown in Fig. 4A, GW9662 or GGPP in combination with lycopene effectively reversed the antiproliferative effect of lycopene and restored lycopene-induced expression of PPARγ and LXRα in DU145 cells (Fig. 4B). In addition, GW9662 or GGPP in combination with lycopene markedly increased the level of cellular total cholesterol and decreased the level of cholesterol in the culture medium, as compared with lycopene (10 μM) treatment group (Fig. 4C).

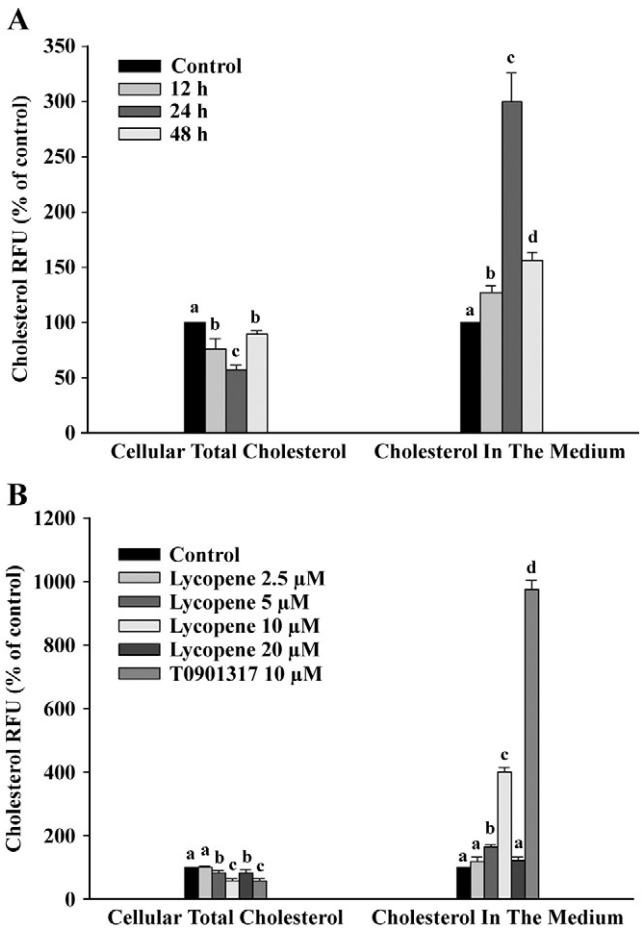

Fig. 3. Effects of lycopene on the levels of cellular total cholesterol and cholesterol in the medium of DU145 cells. (A) Time course effect (incubation with 10 μM lycopene for 12, 24 and 48 h); (B) concentration effect (incubation with 0–20 μM lycopene for 24 h). Values are means±S.D., $n=3$; means not sharing a letter differ significantly ($P<.05$).

3.4. PPARγ-LXRα-ABCA1 pathway participates in the antiproliferative effects of lycopene in DU145 cells

We further employed the si-RNA technique to confirm the role of LXRα in the antiproliferative effect of lycopene. The introduction of si-LXRα to DU145 cells decreased the expression of LXRα and ABCA1 without affecting PPARγ expression (Fig. 5A). In addition, DU145 cells were pretreated with si-LXRα for 48 h and then incubated with lycopene for 24 h to investigate the role of LXRα in the antiproliferative effects of lycopene in DU145 cells. The results reveal that LXRα knockdown significantly increased cell proliferation. In addition, si-LXRα+lycopene treatment restored the cell proliferation to the control level (Fig. 5B), indicating the involvement of LXRα in the antiproliferative action of lycopene in DU145 cells.

3.5. Synergistic effect of lycopene in combination with T0901317 on cell proliferation and protein expression of PPARγ, LXRα and ABCA1

We then combined lycopene (2.5 and 5 μM) with T0901317 (2 μM) to examine the synergistic effect on cell proliferation and protein expression of PPARγ, LXRα and ABCA1. As illustrated in Table 1, lycopene alone (2.5 and 5 μM) concentration-dependently inhibited cell proliferation (by 1.5%, $P=.645$ and 8.3%, $P=.017$, respectively), whereas T0901317 alone (2 μM) significantly inhibited cell proliferation by 13.5% ($P<.05$). The combination of lycopene and T0901317, i.e., 2.5 μM lycopene+2 μM T0901317 and 5 μM lycopene+2 μM T0901317, significantly inhibited cell proliferation (by 38%, $P<.001$ and 46.5%, $P<.001$, respectively), and the results were synergistic (2.6-fold and 2.1-fold of the control, respectively).

As shown in Tables 2–4, lycopene alone (2.5 and 5 μM) significantly increased the protein expression of PPARγ, LXRα and ABCA1, whereas T0901317 alone (2 μM) did not significantly affect protein expression of PPARγ and LXRα, although this agonist significantly increased ABCA1 expression (85%, $P<.001$). The combination of lycopene and T0901317, i.e., 2.5 μM lycopene+2 μM T0901317 and 5 μM lycopene +2 μM T0901317, strongly increased protein expression of PPARγ (3.2-fold, $P=.004$ and 2.0-fold, $P=.003$, respectively), LXRα (4.7-fold, $P=.001$ and 3.6-fold, $P=.002$, respectively) and ABCA1 (2.6-fold, $P=.003$ and 1.8-fold, $P=.002$, respectively).

4. Discussion

Peroxisome proliferator-activated receptor γ and LXRα heterodimerize with retinoid X receptor (RXR) [34], and activation of PPARγ/RXR and LXRα/RXR heterodimer has been shown to inhibit the proliferation of prostate cancer cells [23,25,28,35]. Most recently, we demonstrated that lycopene can inhibit the proliferation of androgen-dependent human prostate tumor cells (LNCaP) through the activation of PPARγ-LXRα-ABCA1 pathway [30]. However, it is still unclear whether lycopene possesses similar effects in androgen-independent prostate cancer cells (DU145 and PC-3). In the present study, we demonstrated that the antiproliferative activity of lycopene in DU145 cells was associated with up-regulating the PPARγ-LXRα-ABCA1 pathway, leading to increased cholesterol efflux. We also

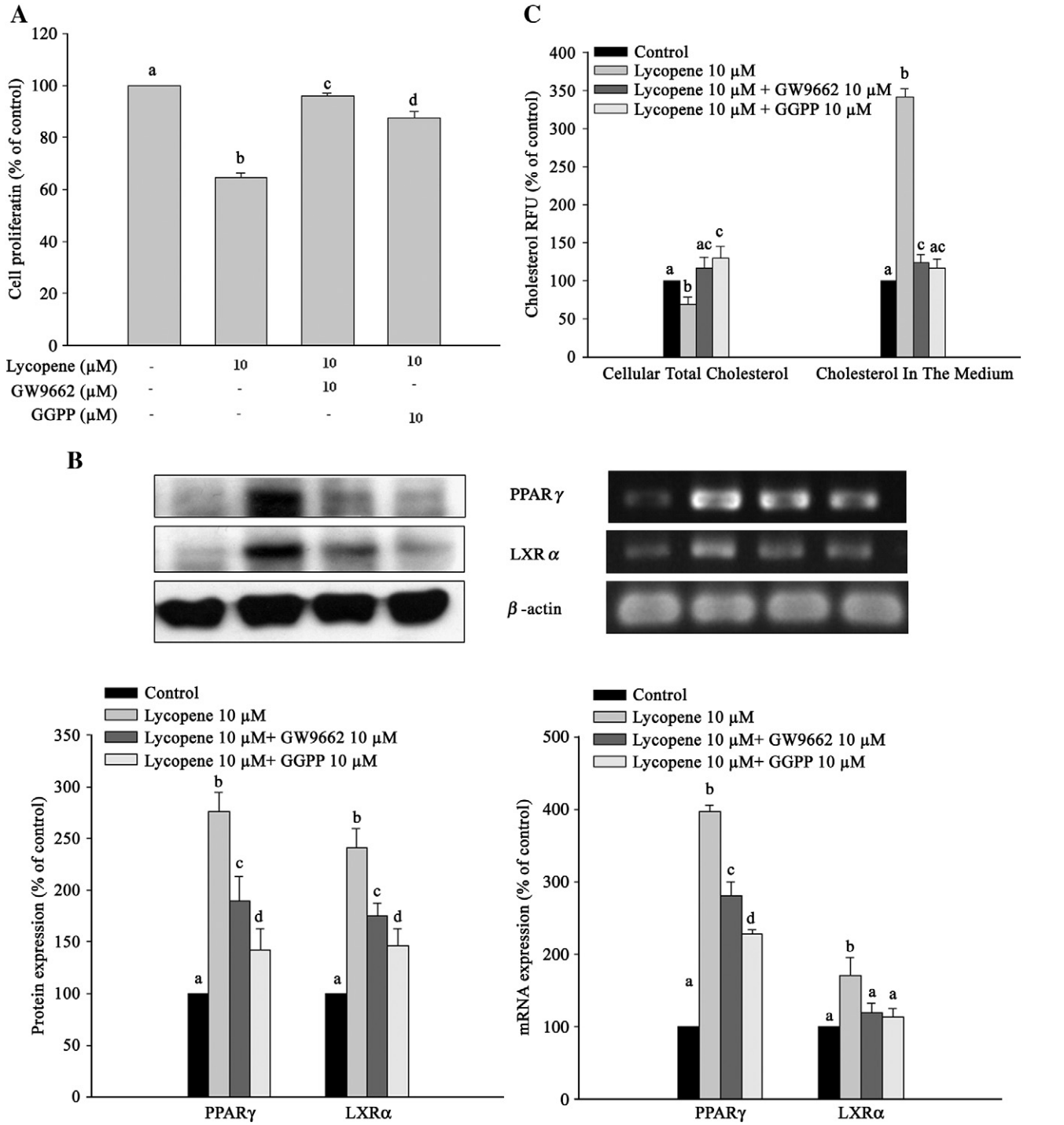

Fig. 4. Cell proliferation (A), protein expression of PPARγ and LXRα (B) and the levels of cellular total cholesterol and cholesterol in the medium (C) in DU145 cells incubated for 24 h with lycopene (10 μM) in the presence or absence of an antagonist of PPARγ (GW9662, 10 μM) or LXRα (GGPP, 10 μM). Values (means±S.D., $n=3$) not sharing a letter differ significantly ($P<.05$).

found that lycopene and T0901317 synergistically inhibited the cell proliferation and activated PPARγ-LXRα-ABCA1 pathway.

Higher cholesterol accumulation in malignancy tumor tissues than that in normal tissues indicates that cholesterol accumulation plays a vital role in cancer progression [36,37]. Abnormal cholesterol metabolism also promotes the growth of prostate cancer [36]. Intracellular cholesterol efflux from peripheral cells to the liver is the first step of the reverse cholesterol transport (RCT) process [38]. Furthermore, ABCA1, belong to the ATP-binding cassette transporter family [39], play a critical role in mediating cellular cholesterol efflux and starting the RCT process [40]. A great deal of evidence indicates that the expression of ABCA1 is mediated through PPARγ-LXRα pathway [40]. The expression of LXRα and ABCA1 was reduced in prostate cancer progression; on the other hand, overexpression of ABCA1 could inhibit proliferation of prostate cancer cells [28,41]. The PPARγ-LXRα-ABCA1 pathway is also involved in cholesterol efflux in macrophage [42]. Our findings that lycopene increased the expression of ABCA1 and increased cholesterol efflux further demonstrate that

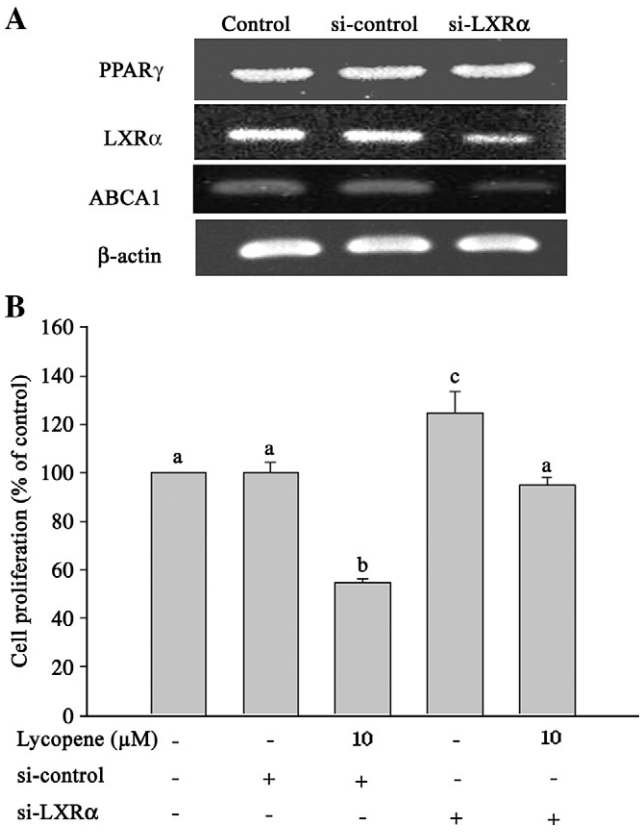

Fig. 5. Peroxisome proliferator-activated receptor γ, LXRα and ABCA1 mRNA expression levels (A) and cell proliferation (B) after knockdown of LXRα. DU145 cells were transfected with si-RNA against LXRα for 48 h, and mRNA expression was determined using reverse transcriptase PCR. After transfection, the cells were incubated with lycopene (10 μM) for 24 h, and the cell proliferation was determined using the MTT assay. Values (means±S.D., $n=3$) not sharing a letter differ significantly ($P<.05$).

lycopene participates in PPARγ-LXRα-ABCA1 pathway by mediating the cholesterol efflux in DU145 cells.

We then used the antagonists and siRNA against LXRα to confirm the involvement of the PPARγ-LXRα-ABCA1 pathway in the anti-proliferative effect of lycopene in DU145 cells. We show that the antagonist of PPARγ (GW9662) and LXRα (GGPP) reversed the antiproliferative effect of lycopene and attenuated lycopene-induced expression of PPARγ, LXRα and ABCA1 in DU145 cells. Using the siRNA technology, we found that si-LXRα inhibited the expression of LXRα and ABCA1 without affecting the expression of PPARγ in DU145 cells, confirming that LXRα is a target gene for the PPARγ as reported previously [42]. It has been shown that LXRα activation inhibits the proliferation of prostate cancer cells [28]. In contrast, knockdown of ABCA1 expression by RNA interference increases the proliferation of androgen-dependent prostate cancer cells [41]. Thus, the inactivation of LXRα and ABCA1 may increase cell proliferation. We show here that si-LXRα treatment increased the proliferation of DU145 cells, whereas subsequent incubation of DU145 cells with lycopene inhibited si-LXRα-induced cell proliferation to the control levels. The present results obtained from the use of antagonists (GW9662 and GGPP) and LXRα knockdown support that higher LXRα expression correlates to enhance the sensitivity of proliferation inhibition of LNCaP cells [28].

Phytochemicals such as lycopene, genistein, resveratrol and curcumin exhibited no pharmacological toxicity, and these phytochemicals could be used alone or in combination with available drug or used as adjuvant to treat cancer [43,44]. T0901317, the synthetic LXR agonist used as the positive control in our study, has been reported to inhibit the growth of prostate cancer *in vitro* and in nude mice, and this effect is associated with activation of LXR-target gene expression [28,45]. We show here that the low concentrations of lycopene (2.5 and 5 μM) in combination with T0901317 (2 μM) synergistically inhibit proliferation of DU145 cells and increase the protein expression of PPARγ, LXRα and ABCA1. This synergy further confirms the involvement of the PPARγ-LXRα-ABCA1 pathway in the antiproliferative effect of lycopene.

As has been noted previously [12,13], the concentration effects of lycopene on most *in vitro* experiments in the present study were bell-shaped, i.e., the effects of lycopene were all lower at 20 μM than at 10 μM. Consistent with our findings, carotenoids including lycopene and β-carotene were shown to have lowered effectiveness as antioxidants

Table 1
Cell proliferation in DU145 cells incubated with lycopene in combination with T0901317 for 24 h

Treatment	Cell proliferation (%)			Fold of synergy[3]
	Observed[1]	Expected (additive)[2]	*P*	
Control	100[a]	–	–	–
Lycopene 2.5 μM	98.5±4[ab]	–	.645	–
Lycopene 5 μM	91.7±4[bc]	–	.017	–
T0901317 2 μM	86.5±3[c]	–	<.001	–
Lycopene 2.5 μM+T0901317 2 μM	62.1±4[d]	84±5	<.001	2.6
Lycopene 5 μM+T0901317 2 μM	53.5±4[e]	79±4	<.001	2.1

1. Values are means±S.D., $n\geq3$; means without a common letter differ significantly.
2. Expected (or additive) %: calculated by summation (of observed percentage of individual cell proliferation) minus the control.
3. The synergy of data is calculated as [(lycopene+T0901317)−control]÷[(lycopene−control)+(T0901317−control)].

Table 2
PPARγ protein expression in DU145 cells incubated with lycopene in combination with T0901317 for 24 h

Treatment	PPARγ protein expression (%)			Fold of synergy[3]
	Observed[1]	Expected (additive)[2]	*P* value	
Control	100[a]	–	–	–
Lycopene 2.5 μM	126±4[b]	–	.11	–
Lycopene 5 μM	159±13[c]	–	<.001	–
T0901317 2 μM	114±13[a]	–	.113	–
Lycopene 2.5 μM+T0901317 2 μM	227±24[d]	140±9	.004	3.2
Lycopene 5 μM+T0901317 2 μM	245±17[d]	173±8	.003	2.0

1. Values are means±S.D., $n\geq3$; means without a common letter differ significantly.
2. Expected (or additive) %: calculated by summation (of observed percentage of individual protein expression) minus the control.
3. The synergy of data is calculated as [(lycopene+T0901317)−control]÷[(lycopene−control)+(T0901317−control)].

Table 3
LXRα protein expression in DU145 cells incubated with lycopene in combination with T0901317 for 24 h

Treatment	LXRα protein expression (%)			Fold of synergy[3]
	Observed[1]	Expected (additive)[2]	*P* value	
Control	100[a]	–	–	–
Lycopene 2.5 μM	125±7[bc]	–	.036	–
Lycopene 5 μM	143±15[c]	–	.003	–
T0901317 2 μM	121±18[a]	–	.068	–
Lycopene 2.5 μM+T0901317 2 μM	317±12[d]	146±25	<.001	4.7
Lycopene 5 μM+T0901317 2 μM	329±8[d]	164±32	.002	3.6

1. Values are means±S.D., $n\geq3$; means without a common letter differ significantly.
2. Expected (or additive) %: calculated by summation (of observed percentage of individual protein expression) minus the control.
3. The synergy of data is calculated as [(lycopene+T0901317)−control]÷[(lycopene−control)+(T0901317−control)].

Table 4
ABCA1 protein expression in DU145 cells incubated with lycopene in combination with T0901317 for 24 h

Treatment	ABCA1 protein expression (%)			Fold of synergy[3]
	Observed[1]	Expected (additive)[2]	*P* value	
Control	100[a]	–	–	–
Lycopene 2.5 μM	121±16[a]	–	.143	–
Lycopene 5 μM	148±3[b]	–	.006	–
T0901317 2 μM	185±27[c]	–	<.001	–
Lycopene 2.5 μM+T0901317 2 μM	373±38[d]	205±27	.003	2.6
Lycopene 5 μM+T0901317 2 μM	338±13[d]	232±30	.002	1.8

1. Values are means±S.D., $n \geqq 3$; means without a common letter differ significantly.
2. Expected (or additive) %: calculated by summation (of observed percentage of individual protein expression) minus the control.
3. The synergy of data is calculated as [(lycopene+T0901317)−control]÷[(lycopene−control)+(T0901317−control)].

and anticarcinogens *in vitro* at concentrations >10 μM [46,47]. A possible explanation for the bell-shaped effects is that the antioxidant activity of carotenoid may shift into prooxidant activity, depending on carotenoid concentration inside the cells and cell redox status [48]. As compared with physiological plasma lycopene concentrations around 0.5 μM [8], lycopene concentrations (2.5–20 μM) used in this study are relatively high. However, plasma levels of lycopene can be increased markedly by lycopene supplementation. For example, plasma levels of lycopene were increased from 428 to 960 nM after 18.4 mg/d lycopene from 240 g (~1 cup/d) of tomato juice for 3-week supplementation [49]. Thus, the concentrations of lycopene used in the present study are of pharmacological relevance.

Notably, the activation time of PPARγ, LXRα and ABCA1 expression and cholesterol efflux is different between the androgen-dependent LNCaP cells [30] and the androgen-independent DU145 cells, i.e., the strongest activation time was 24 h for PPARγ and LXRα and 96 h for ABCA1 and cholesterol efflux in LNCaP cells [30], whereas the strongest activation time was 24 h for PPARγ, LXRα and ABCA1 expression and cholesterol efflux in DU145 cells. A possible explanation is the different doubling time between LNCaP cells (48–60 h) [30,50] and DU145 cells (24 h). In addition, LNCaP and DU145 cell lines are different in growth factor production and hormone sensitivity. In this respect, DU145 cells generate higher levels of several growth factors including epidermal growth factor, transforming growth factor and basic fibroblast growth factor than do the LNCaP cells [51]. In addition, androgens affect the growth of LNCaP cells but not DU145 cells [51]. Apparently, the activation of PPARγ-LXRα-ABCA1 pathway by lycopene is independent of the androgen sensitivity of prostate cancer cells.

We have previously shown that the stability of lycopene varies with different delivery vehicles (micelles>THF/BHT-FBS>methyl-β-cyclodextrin>THF/BHT>THF) and decreases with increased incubation time. After incubation for 24 h, approximately 50% lycopene remains in the medium when THF/BHT-FBS was used as the delivery vehicle [31]. In addition, we showed that lycopene delivered by THF/BHT-FBS is indeed taken up by the prostate cancer DU145 and PC-3 cells [31]. Therefore, our present data suggest that the activation of PPARγ-LXRα-ABCA1 pathway by lycopene may not be due to lycopene itself but likely to its oxidation products or both. However, our main findings support and strengthen the new notion that lycopene (or its metabolic products) can exert its antiproliferative effect on prostate cancer cells, either androgen-dependent (LNCaP) cells or androgen-independent (DU145 and PC-3) cells, through the PPARγ-LXRα-ABCA1 pathway.

In summary, the present findings have clearly indicated that the antiproliferative action of lycopene involves the activation of PPARγ-LXRα-ABCA1 pathway, leading to enhanced cholesterol efflux in androgen-independent prostate cancer cells. In addition, the synergy of lycopene and T0901317 on antiproliferation of DU145 cells suggests that lycopene may be used as adjuvant to enhance the antiproliferative effect of T0901317.

Supplementary materials related to this article can be found online at doi:10.1016/j.jnutbio.2011.06.009.

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